J. Pharm. Pharmac., 1969, **21**, 662–667 *Received May* 15, 1969

The actions of atropine, tropenziline and *N*-butyl hyoscine bromide on the isolated distal colon of the guinea-pig: a comparison of their activities and mechanisms of action

S. LECCHINI, M. DEL TACCA, G. SOLDANI, G. M. FRIGO AND A. CREMA

Department of Pharmacology, University of Pisa, Italy

In the guinea-pig isolated distal colon, the order of anticholinergic activity is as follows: atropine > tropenziline bromide > *N*-butyl hyoscine bromide. The reduction in the responses to pelvic and transmural stimulation produced by tropenziline and *N*-butyl hyoscine bromide is due partly to their ganglion blocking activity. This effect also explains the reduction they cause in acetylcholine output on pelvic nerve and transmural stimulation. Since atropine also reduces acetylcholine release on pelvic nerve stimulation, it is suggested that muscarinic receptors of the parasympathetic ganglia are involved in transmission of pelvic nerve impulses.

A number of investigations have been made on the mammalian colon to analyse quantitatively the blocking effect of anticholinergic drugs on endogenously released and exogenous acetylcholine.

Studies with atropine have been made by Munro (1953), Garry & Gillespie (1955), Lee (1960), Gillespie & MacKenna (1960), Fülgraff & Schmidt (1964), Rand & Ridehalgh (1965) and Campbell (1966). According to Rothlin, Taeschler & others (1954) and Taeschler, Konzett & Cerletti (1960) tropenziline bromide interferes with nervous transmission in the vagal plexus of the intestine and thus exerts a spasmolytic effect, which has also been confirmed clinically by Kewenter, Kock & others (1962) and by Shah, Shet & Shah (1964). The action of *N*-butyl hyoscine bromide on the intestinal tract has been investigated by Wick (1951), who found that it was more active on the intestine than was atropine in blocking the effects produced by vagal stimulation, leading to the conclusion that *N*-butyl hyoscine bromide has strong ganglion-blocking properties. The ganglion blocking activity of *N*-butyl hyoscine bromide has been demonstrated by Holtz & Schümann (1956), Herman, Shaw & Rosenblum (1958), and Pennefather, McCulloch & Rand (1968).

In this paper we deal with comparisons of the activities of atropine sulphate, 6-methoxytropine benzilate-*N*-methylbromide (tropenziline) and *N*-butyl hyoscine bromide on the responses of guinea-pig colon to acetylcholine, and to pelvic and transmural stimulation. Moreover, as there is no direct proof of the existence of a ganglion blocking activity of tropenziline or *N*-butyl hyoscine bromide at the level of the parasympathetic ganglia of the intestinal plexuses, we intend to investigate this problem by examining the effects of these drugs on the amount of acetylcholine released by pelvic nerve stimulation.

EXPERIMENTAL

Guinea-pig isolated colon was prepared similarly to the rabbit preparation described by Garry & Gillespie (1955).

The terminal colon, length 3–4 cm, together with the pelvic nerves was removed from 150 adult female guinea-pigs, 250–300 g. Bipolar electrodes, made with rings of silver wire (2 mm apart), were placed around the nerves. Transmural stimulation was applied by inserting a silver wire into the lumen and by placing the reference electrode into the bath. The preparation was suspended vertically in a 10 ml bath containing an oxygenated Tyrode solution at 35°. The contractions of the longitudinal muscle were recorded with a light frontral isotonic gimbal lever, exerting a tension of 2·5 g. The preparation was left in the bath for about 120 min before beginning the experiment. To stimulate the pelvic and intramural nerves, trains of rectangular pulses with 1 ms pulse duration and a frequency of 10 Hz were applied for 30 s. The pelvic nerve was stimulated supramaximally. The strength of the transmural stimulation (20 V) and the acetylcholine concentration (1×10^{-7} g/ml) were selected to give a response similar to that produced by pelvic nerve stimulation.

For the construction of dose-response curves in each experiment, the activity of an anticholinergic drug was evaluated against one kind of stimulation. Progressively increasing concentrations of drugs were added to the bath at 30 min intervals, each addition being 20 min before stimulation. Since the response to transmural stimulation after atropine became biphasic or triphasic according to the drug concentration used, the height of contraction above the base line was chosen for measurement and was compared with the height of the control contraction (Del Tacca, Lecchini & others, 1968). The heights of contractions produced by acetylcholine, pelvic and transmural stimulation after drug treatment were expressed as percentages of the control contractions to calculate the ED50 values.

For estimating acetylcholine release and the effects of drugs on it, the colon was prepared as described above, and was then incubated for 40 min with physostigmine sulphate (1×10^{-5} g/ml). Concentrations of atropine, tropenziline and *N*-butyl hyoscine bromide causing a reduction of about 60% of the response to pelvic and transmural stimulation were chosen.

The experiments were made as follows: during the first period the preparation was stimulated (pelvic or transmural stimulation) for 10 min, and then was left to rest for 10 min, after which time the Tyrode solution was removed. During the second period, after washing, the preparation was kept at rest for 20 min; then the Tyrode solution was again removed. The first and second periods were taken as a trial cycle, which was repeated 3 times. The first cycle was discarded and the second cycle was used as a control: between the second and the third cycle the drugs were added to the colon. The acetylcholine content in the samples (diluted 1 to 1·2 with distilled water) was assayed against suitable standards on frog rectus abdominis muscle added with 5×10^{-6} g/ml physostigmine sulphate. The amount of acetylcholine released during a 10 min resting period was subtracted from that released during the period of stimulation to obtain the quantity released during the 10 min period of stimulation.

The following drugs were used: atropine sulphate, 6-methoxytropine benzilate-*N*-methylbromide (tropenziline), *N*-butyl hyoscine bromide, acetylcholine chloride, hexamethonium bromide and physostigmine sulphate. The concentrations are expressed in terms of base.

RESULTS

In 13 out of 67 preparations the responses to pelvic and transmural stimulation, but not those to exogenous acetylcholine, were partially resistant to the blocking actions of atropine, tropenziline and *N*-butyl hyoscine bromide, as had also been previously noted for atropine by Del Tacca & others (1968). The resistant preparations were not included in the calculation of the ED50.

Table 1 shows that the potency of blocking activity against both endogenous and exogenous acetylcholine is in the order: atropine > tropenziline > *N*-butyl hyoscine bromide. Atropine and tropenziline were significantly more active in blocking responses to exogenous acetylcholine than in blocking those to either transmural or pelvic stimulation. For *N*-butyl hyoscine bromide, activity against responses to pelvic stimulation, but not that to transmural stimulation, was significantly different from its activity against exogenous acetylcholine. Atropine reduced the response to transmural stimulation at lower concentrations than those necessary to reduce the response to pelvic stimulation. On the other hand, the response to pelvic and transmural stimulation were inhibited by concentrations of tropenziline and *N*-butyl hyoscine bromide which were not significantly different. For all three substances there was a linear relation between log dose and effect.

The amounts of acetylcholine released from the colon vary considerably, not only from one species to another, but also with preparations of colon from a single species.

Table 1. *ED50 and slopes* (b) *of regression lines with 95% fiducial limits of atropine, tropenziline and N-butyl hyoscine bromide on the contraction producted by acetylcholine* (1×10^{-7} g/ml), *transmural* (10 Hz) *and pelvic* (10 Hz) *stimulation in normal guinea-pig colon. The probability values (P) for the significance of differences between ED50 within groups are given.*

Drugs	No. of expts	Stimulating agents	ED50 with fiducial limits (95%)	b values with fiducial limits (95%)	*P* values
Atropine	8	Acetylcholine (ACh)	$2{\cdot}59 \times 10^{-9}$ M $\{3{\cdot}63 \times 10^{-9}$ / $1{\cdot}91 \times 10^{-9}$	$73{\cdot}13\{69{\cdot}96$ / $76{\cdot}30$	ACh/P < 0·001
	8	Transmural (T)	$5{\cdot}41 \times 10^{-9}$ M $\{8{\cdot}78 \times 10^{-9}$ / $3{\cdot}45 \times 10^{-9}$	$63{\cdot}21\{56{\cdot}20$ / $70{\cdot}22$	ACh/T < 0·01
	8	Pelvic (P)	$9{\cdot}95 \times 10^{-9}$ M $\{2{\cdot}64 \times 10^{-8}$ / $6{\cdot}50 \times 10^{-9}$	$41{\cdot}39\{35{\cdot}85$ / $46{\cdot}93$	PS/T < 0·05
Tropenziline	7	Acetylcholine (ACh)	$1{\cdot}57 \times 10^{-8}$ M $\{2{\cdot}56 \times 10^{-8}$ / $1{\cdot}04 \times 10^{-8}$	$57{\cdot}99\{52{\cdot}87$ / $63{\cdot}11$	ACh/P < 0·02
	10	Transmural (T)	$2{\cdot}83 \times 10^{-8}$ M $\{4{\cdot}49 \times 10^{-8}$ / $1{\cdot}88 \times 10^{-8}$	$54{\cdot}73\{51{\cdot}76$ / $57{\cdot}69$	ACh/T < 0·05
	10	Pelvic (P)	$3{\cdot}54 \times 10^{-8}$ M $\{6{\cdot}21 \times 10^{-8}$ / $2{\cdot}14 \times 10^{-8}$	$42{\cdot}68\{40{\cdot}45$ / $44{\cdot}91$	PS/T = N.S.
N-Butyl hyoscine bromide	6	Acetychloline (ACh)	$8{\cdot}13 \times 10^{-8}$ M $\{1{\cdot}40 \times 10^{-7}$ / $4{\cdot}74 \times 10^{-8}$	$73{\cdot}51\{73{\cdot}03$ / $73{\cdot}99$	ACh/P < 0·05
	8	Transmural (T)	$1{\cdot}15 \times 10^{-7}$ M $\{2{\cdot}16 \times 10^{-7}$ / $5{\cdot}47 \times 10^{-8}$	$54{\cdot}93\{59{\cdot}44$ / $50{\cdot}41$	ACh/T = N.S.
	10	Pelvic (P)	$2{\cdot}54 \times 10^{-7}$ M $\{6{\cdot}74 \times 10^{-7}$ / $9{\cdot}85 \times 10^{-8}$	$34{\cdot}25\{33{\cdot}78$ / $34{\cdot}73$	PS/T × N.S.

Table 2. *Acetylcholine output from the guinea-pig colon. Action of different anticholinergic drugs on transmural stimulation and resting acetylcholine output* (ng/g/10 min ± s.e.).

Drugs	Concn (M)	Stimulation		Resting	
		Control	After drug	Control	After drug
Atropine (10)*	$8{\cdot}60 \times 10^{-9}$	1·089 ± 0·058	1·185 N.S. ± 0·139	0·301 ± 0·025	0·288 N.S. ± 0·027
Tropenziline (9)*	$3{\cdot}09 \times 10^{-8}$	0·948 ± 0·164	0·750 $P < 0{\cdot}02$ ± 0·160	0·327 ± 0·049	0·303 N.S. ± 0·055
N-Butyl hyoscine bromide (9)*	$2{\cdot}27 \times 10^{-7}$	1·083 ± 0·149	0·891 $P < 0{\cdot}05$ ± 0·144	0·312 ± 0·035	0·276 N.S. ± 0·043

* No. of experiments
P = probability values for significance of difference between the mean amounts of acetylcholine released before and after drug addition. N.S. = no significant difference.

Table 3. *Acetylcholine output from the guinea-pig colon. Action of different anticholinergic drugs on resting and pelvic stimulation acetylcholine output* (ng/g/10 min ± s.e.).

Drugs	Concn (M)	Stimulation		Resting	
		Control	After drug	Control	After drug
Atropine (15)*	$1{\cdot}72 \times 10^{-8}$	0·568 ± 0·056	0·424 $P < 0{\cdot}001$ ± 0·039	0·342 ± 0·040	0·297 N.S. ± 0·029
Tropenziline (6)*	$6{\cdot}29 \times 10^{-8}$	0·746 ± 0·051	0·516 $P < 0{\cdot}01$ ± 0·036	0·385 ± 0·035	0·306 N.S. ± 0·014
N-Butyl hyoscine bromide (6)*	$3{\cdot}27 \times 10^{-7}$	0·616 ± 0·065	0·422 $P < 0{\cdot}02$ ± 0·042	0·281 ± 0·030	0·220 N.S. ± 0·024

* No. of experiments
P = probability values for significance of difference between the mean amounts of acetylcholine released before and after drug addition. N.S. = no significant difference.

We have observed this and it is in agreement with the results obtained by Paton & Vizi (1969). Therefore, the effects of the drugs were evaluated by comparing, in each experiment, the amounts of acetylcholine released before and after drug addition. Beani, Bianchi & Crema (1969) observed that a second period of transmural stimulation resulted in the release of a quantity of acetylcholine not significantly different from that of the first period. In 5 experiments, we found, additionally, that with two periods of pelvic stimulation, the second stimulation caused a release of acetylcholine not signicantly different from the first.

Tables 2 and 3 show that atropine, tropenziline, and *N*-butyl hyoscine bromide did not reduce the resting output of acetylcholine, whereas release during pelvic and transmural stimulation was significantly reduced by tropenziline and *N*-butyl hyoscine bromide. Atropine reduced the output only during pelvic stimulation. Expressed in percentages the amounts released by transmural stimulation were reduced by 21% by tropenziline and by 18% by *N*-butyl hyoscine bromide. The acetylcholine released by pelvic stimulation was reduced by 31% by both tropenziline and *N*-butyl hyoscine bromide. The amount released by pelvic stimulation was reduced by 25% by atropine.

In 2 experiments, hexamethonium (1×10^{-4} g/ml) was used to block ganglion transmission and this resulted in a reduction of 39% of the resting release and of 49% of the amount released by pelvic stimulation.

DISCUSSION

In the colon, atropine exhibited a greater anticholinergic activity than either tropenziline or hyoscine *N*-butyl bromide, in agreement with findings in other organs by Wick (1951), Rothlin & others (1954), Holtz & Schümann (1956), Herman & others (1958), and Pennefather & others (1968). All three parasympatholytic agents antagonized the effects of exogenous acetylcholine more readily than those caused by endogenous acetylcholine.

It has been shown (for reviews see Barlow, 1964; Gyermek, 1967) that the quaternization of tropane derivatives increases ganglion blocking activity and decreases the antimuscarinic potency. In the guinea-pig colon the ratio between the ED50 against responses to pelvic stimulation and the ED50 against responses to exogenous acetylcholine is 4·05 for atropine, 2·25 for tropenziline and 3·12 for *N*-butyl hyoscine bromide. These different ratios could indicate that the quaternary derivatives exert a ganglion blocking action also in the colon. The effects of these drugs on the release of acetylcholine confirm this supposition. In fact, concentrations of tropenziline and *N*-butyl hyoscine bromide which reduce the response to pelvic stimulation by 60%, also cause a 31% reduction of acetylcholine output.

It has been shown that hexamethonium reduces the response to submaximal transmural stimulation (Schaumann, 1956; Härtfelder, Kuschinsky & Mosler, 1958; Kern & Lembeck, 1959; Paton & Vane, 1963; Bianchi, Beani & others, 1968) and that it decreases the acetylcholine output associated with transmural stimulation, lending support to the hypothesis that the ganglia of the colon can act as a multiplier of the submaximal transmural stimulus (Bianchi & others, 1968). In our experiments the strength of transmural stimulation was submaximal and, therefore, signs of the ganglion blocking activity of the quaternary compounds could be expected to appear. However, all three drugs, at the concentrations employed, were ineffective on the resting output of acetylcholine, although it was reduced by hexamethonium, confirming previous results (Beani & others, 1969). No further effort was made to investigate whether or not the action of ganglion blocking drugs on the resting output was dependent upon the concentration employed.

Atropine did not modify the acetylcholine release produced by transmural stimulation, which is in agreement with the findings of Schaumann (1956) and Del Tacca & others (1968), but it significantly reduced its release on pelvic stimulation (a similar but not significant effect was noted in a smaller number of experiments by Del Tacca & others, 1968). An explanation for the effect of atropine in reducing the amount of acetylcholine released by pelvic stimulation is that atropine blocks transmission by acting on muscarinic receptors at the level of the ganglia (for reviews see Volle, 1966; Trendelenburg, 1967). If this explanation is correct it implies the existence of muscarinic receptors on parasympathetic ganglion cells, which is at variance with the conclusions of Roszkowski (1961) and Smith (1966). It is possible that physostigmine reveals the presence of muscarinic receptor, as was the case with both the sympathetic and parasympathetic ganglia in which the ganglion blocking action of atropine was unmasked by the presence of anticholinesterase drugs (Flacke & Gillis, 1968), as well as

by the continuous perfusion of acetylcholine (Gebber & Snyder, 1968). Further experiments are needed to investigate the action of atropine on parasympathetic ganglia.

In conclusion, tropenziline and *N*-butyl hyoscine bromide have two sites of action in the guinea-pig colon. In addition to their principal antimuscarinic activity on the smooth muscle, they have a ganglion blocking action exerted through nicotinic receptors and, possibly, also through muscarinic receptors.

REFERENCES

BARLOW, R. B. (1964). *Introduction to Chemical Pharmacology*, London: Methuen & Co. Ltd.

BEANI, L., BIANCHI, C. & CREMA, A. (1969). *Br. J. Pharmac.*, **36**, 1–17.

BIANCHI, C., BEANI, L., FRIGO, G. M. & CREMA, A. (1968). *Europ. J. Pharmac.*, **4**, 51–61.

CAMBPELL, G. (1966). *J. Physiol., Lond.*, **185**, 148–159.

DEL TACCA, M., LECCHINI, S., FRIGO, G. M., CREMA, A. & BENZI, G. (1968). *Europ. J. Pharmac.*, **4**, 188–197.

FLACKE, W. & GILLIS, R. A. (1968). *J. Pharmac. exp. Ther.*, **163**, 266–276.

FÜLGRAFF, G. & SCHMIDT, L. (1964). *Archs int. Pharmacodyn. Thér.*, **149**, 552–559.

GARRY, R. C. & GILLESPIE, J. S. (1955). *J. Physiol., Lond.*, **128**, 557–576.

GEBBER, G. L. & SNYDER, D. W. (1968). *J. Pharmac. exp. Ther.*, **163**, 64–74.

GILLESPIE, J. S. & MCKENNA, B. R. (1960). *J. Physiol., Lond.*, **152**, 191–205.

GYERMEK, L. (1967). *In Drugs affecting the Peripheral Nervous System*, Editor: Burger, A. Vol. 2, pp. 149–326, New York: M. Dekker, Inc.

HÄRTFELDER, G., KUSCHINSKY, G. & MOSLER, K. H. (1958). *Arch. exp. Path. Pharmak.*, **234**, 66–78.

HERMANN, L., SHAW, F. H. & ROSENBLUM, E. I. (1958). *J. Pharm. Pharmac.*, **10**, 348–355.

HOLTZ, P. & SCHÜMANN, H. J. (1956). *Arch. exp. Path. Pharmak.*, **229**, 101–112.

KERN, H. & LEMBECK, F. (1959). *Ibid.*, **236**, 325–330.

KEWENTER, J., KOCK, N. G., POMPEIUS, R. & SCHELLER, S. (1962). *Acta chir. scand.*, **123**, 396–404.

LEE, C. Y. (1960). *J. Physiol., Lond.*, **152**, 405–418.

MUNRO, A. F. (1953). *Ibid.*, **120**, 41–52.

PATON, W. D. M. & VANE, J. R. (1963). *Ibid.*, **165**, 10–46.

PATON, W. D. M. & VIZI, E. S. (1969). *Br. J. Pharmac.*, **35**, 10–28.

PENNEFATHER, J. N., MCCULLOCH, M. W. & RAND, M. J. (1968). *J. Pharm. Pharmac.*, **20**, 867–872.

RAND, M. J. & RIDEHALGH, A. (1965). *Ibid.*, **17**, 144–156.

ROSZKOWSKI, A. P. (1961). *J. Pharmac. exp. Ther.*, **132**, 156–170.

ROTHLIN, E., TAESCHLER, M., KONZETT, H. & CERLETTI, A. (1954). *Experentia*, **10**, 142–145.

SCHAUMANN, W. (1956). *Nature, Lond.*, **178**, 1121.

SHAH, N. R., SHETH, R. D. & SHAH, R. C. (1964). *J. Ass. Phyns India*, **12**, 771–775.

SMITH, J. C. (1966). *J. Pharmac. exp. Ther.*, **153**, 266–275.

TAESCHLER, M., KONZETT, H. & CERLETTI, A. (1960). *Schweiz. Med. Wschr.*, **90**, 136–140.

TRENDELENBURG, U. (1967). *Ergerb. Physiol.*, **59**, 2–85.

VOLLE, R. L. (1966). *Pharmac. Rev.*, **18**, 839–869.

WICK, H. (1951). *Arch. exp. Path. Pharmak.*, **213**, 485–500.